Structure
Previews

Entry of Herpesviruses into Cells: More than One Way to Pull the Trigger

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Interaction of viral protein gp42 with its receptor is the trigger for the entry of Epstein-Barr virus into B cells. The structure of gp42 reported by Kirschner et al. (2009) in this issue of *Structure* suggests a likely triggering mechanism substantially different from that of a related herpesvirus.

To deliver its genetic material into a cell, any enveloped virus must fuse its lipid envelope with the lipid membrane of the target cell. Membrane fusion by herpesviruses is complex and requires the action of three conserved proteins: gB and gH/gL complex. Some herpesviruses employ additional glycoproteins to bind cell-surface receptors and trigger membrane fusion. Given that herpesviruses use different triggers to activate conserved fusion machinery, a big dilemma in the field is whether these triggers act differently or not.

Perhaps the best-studied triggering mechanism of herpesvirus fusion is that of Herpes Simplex virus Type 1 (HSV-1), which uses gD as a receptor-binding protein. From extensive characterization of the binding of gD to its receptors, we know that the flexible C terminus of gD is sequestered at the gD dimer interface in the absence of a receptor and occludes the receptor-binding site (Figure 1A). Receptor binding releases the C terminus, which is thought to trigger fusion, for which gB and gH/gL are required (Figure 1A) (Fusco et al., 2005; Krummenacher et al., 2005).

Entry of Epstein-Barr virus (EBV) into B cells requires the interaction of viral protein gp42 with human leukocyte activator (HLA), an MHC class II molecule. In a yet unknown manner, this interaction triggers membrane fusion carried out by gB and gH/gL. In this capacity, EBV gp42 has been considered to be a functional homolog of HSV gD; there is no sequence similarity between the two proteins. The current work of Kirschner and colleagues (Kirschner et al., 2009) builds upon the group's previous results to suggest that the mechanisms by which gp42 and gD trigger membrane fusion may be fundamentally different.

The same group previously determined the structure of the gp42 ectodomain bound to its receptor HLA-DR1 (Mullen et al., 2002). It showed that gp42 consists of a C-terminal lectin-like domain (CTLD) and an N-terminal extension. This structure also revealed the location of the HLA-binding site and highlighted a hydrophobic pocket on the surface of gp42. Follow-up mutagenesis generated hydrophobic pocket mutants that maintained wild-type HLA binding but could no longer trigger fusion, which emphasized the functional significance of the hydrophobic pocket in gp42 (Silva et al., 2004).

gp42 forms a complex with gH/gL, and a different series of experiments mapped the location of the gH/gL-binding site to N-terminal residues 36–81 (Kirschner et al., 2007). This region was, unfortunately, disordered in the gp42/HLA structure, but the linker connecting the CTLD and the gH/gL-binding site could be seen. Thus, gp42 contains three important functional sites: an HLA-binding site within CTLD; a hydrophobic pocket within CTLD; and a gH/gL-binding site on the N-terminal extension.

From the structure of gp42/HLA complex only (Mullen et al., 2002), it was unclear how receptor binding could trigger the fusion machinery. The structure of unbound gp42 reported here (Kirschner et al., 2009) finally allows for a comparison to identify HLA-induced conformational changes in gp42. Whereas the HLA-binding site remains unaltered and the gH/gL-binding site is still disordered, changes do occur in the hydrophobic pocket and the N-terminal linker. These conformational differences lead the authors to propose a plausible model of the role of gp42 in triggering membrane fusion.

The first important conclusion from comparing the structures of the unbound and HLA-bound gp42 is that the hydrophobic pocket is slightly wider in the HLA-bound gp42. Moreover, this conformational change appears to propagate from the HLA-binding site, suggesting that the binding of HLA is what broadens the hydrophobic pocket. A broader hydrophobic pocket could enable binding of a large ligand. One possibility is that the hydrophobic pocket could be a part of a bidentate binding site for gH/gL, along with the N terminus (Figure 1B). Yet, the known N-terminal gH/gL-binding site is far from the hydrophobic pocket, plus fusion-inactive mutants of the hydrophobic pocket bind gH/gL with wild-type affinity (Silva et al., 2004). Thus, another possibility is that the hydrophobic pocket could recruit gB, another protein required for fusion. If gp42 can, indeed, simultaneously bind both gH/gL and gB, this would imply assembly of a fusogenic complex (Figure 1B). For comparison, in the presence of its receptor, HSV-1 gD promotes the interaction between gB and gH/gL, thereby triggering fusion (Atanasiu et al., 2007; Avitabile et al., 2007). However, the formation of a complex between gD and gH/gL or gD and gB has not been observed directly.

The second important conclusion from the comparison of gp42 and gp42/HLA structures is that the N-terminal linker, residues 87–93, adopts two different conformations. Although residues 87–93 themselves are not important for gH/gL binding, 5-residue deletions within this region block fusion, which supports a critical role for this short amino-acid stretch in gp42. More interestingly, in gp42 crystals, N-terminal residues 87–93 contact neighboring molecules at the HLA-binding sites (Figure 1B, left). The presence of two such nearly identical, crystallographically independent interactions is unlikely to be

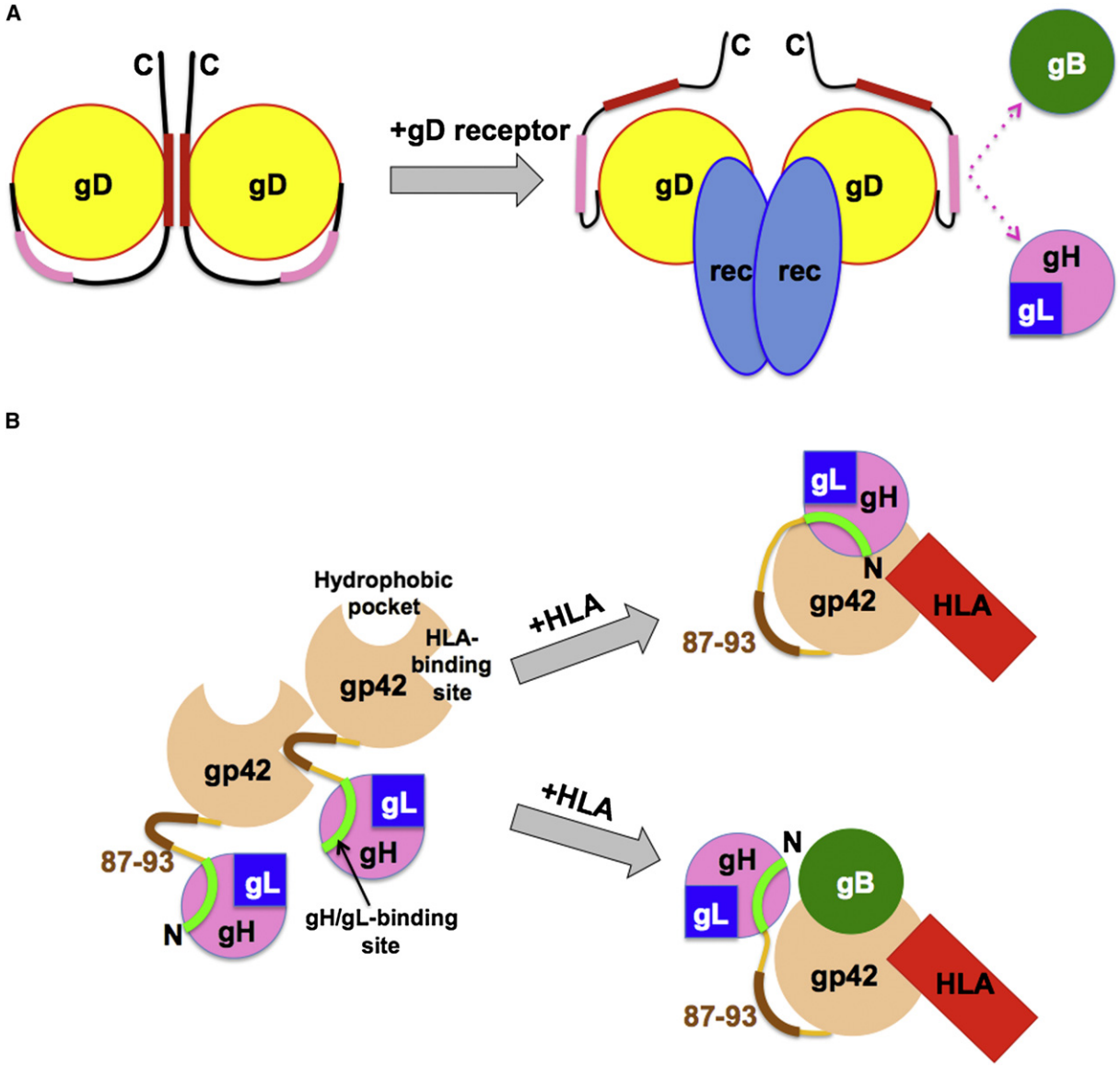

Figure 1. Receptor-Induced Conformational Changes in gD and gp42

(A) Conformational changes in gD upon receptor binding (adapted from Krummenacher et al., 2005). The globular receptor-binding domain of gD is shown in yellow. The flexible C-terminal extension in gD is shown in black, red, and pink. A part of the C terminus (red) is sequestered at a gD dimer interface in the absence of receptor. Binding of a receptor releases the C terminus. Another region in the C terminus (pink) is thought to trigger fusion, presumably by signaling to gB or gH/gL or both.

(B) A model of conformational changes in gp42 upon receptor binding, based on work reported by Kirschner et al. (2009). The CTLD of gp42 is shown in buff, and the N-terminal extension is shown in orange. The gH/gL-binding site (residues 36–81) is shown in green, and the linker connecting the gH/gL-binding site to the CTLD (residues 87–93) is shown in brown. In the absence of a receptor, residues 87–93 of one gp42 molecule contact the HLA-binding site of a neighboring molecule. Binding of HLA would displace residues 87–93, and their movement would cause bound gH/gL to relocate. A wider hydrophobic pocket allows either gH/gL (top) or gB (bottom) to bind.

an artifact. Therefore, residues 87–93 may have an affinity for the HLA-binding site. It is tempting to speculate that interactions involving the N terminus of one gp42 molecule with the HLA-binding site of another are biologically relevant. Kirschner and colleagues propose that binding of HLA would displace residues 87–93 from the HLA-binding site and provide a change in structure that is important to trigger fusion. Indeed, one could envision a scenario in which, once displaced by HLA, residues 87–93 could function as a hinge that repositions gH/gL relative to gp42, thereby promoting fusion.

The interactions between N-terminal residues 87–93 and the HLA-binding sites form an extended polymer-like network of gp42 molecules in the crystals. Based on this, Kirschner and colleagues speculate about the potential for gp42 molecules to be linked on the viral surface into a 2D network. Upon HLA binding, this network would become disturbed; the subsequent rearrangements of glycoproteins could be a part of the triggering mechanism. Ordered arrangements of viral proteins

on the viral envelope have been seen in unrelated viruses (e.g., flaviviruses [Mukhopadhyay et al., 2005]). Although such 2D networks have not been identified on the envelopes of herpesviruses, cryoelectron tomography images of HSV-1 show local clustering of viral proteins (Grunewald et al., 2003). Perhaps a similar imaging of EBV virions will reveal ordered envelope glycoproteins and confirm this interesting hypothesis.

From these and previous data, one could derive the following model (Figure 1B). On the viral surface, gp42 is in complex with gH/gL, bound to residues 36–81; the linker connecting the gH/gL-binding site to the CTLD, residues 87–93, is bound to the HLA-binding site of a neighboring molecule. When HLA binds gp42, it would displace residues 87–93 and cause the hydrophobic pocket to widen. The changed conformation of residues 87–93 would then force the bound gH/gL to relocate. At the same time, the wider hydrophobic pocket could accommodate either gH/gL or gB. Although this model is largely speculative, it suggests new directions for probing the complex mechanism of EBV entry.

The authors compare this potential mechanism to that of HSV-1 gD. They note that just as receptor binding releases the HSV-1 gD C terminus, HLA binding releases N-terminal residues 87–93 of EBV gp42. The similarities may end here, however, because the triggering mechanism of gp42 has many differences with that of HSV-1 gD. For example, a stable gD/gH/gL complex has not yet been observed, but gp42 forms a ternary complex with gH/gL even in the absence of its receptor. Furthermore, although receptor binding frees the gD C terminus to interact with gH/gL or gB or both, residues 87–93 of gp42 are not involved in binding gH/gL. This region may act more like a hinge to reposition the gH/gL complex that remains bound upstream. More work is necessary to reveal the detailed mechanism of triggering in both EBV and HSV-1. For now, all that is certain is that the triggering of membrane fusion during the entry of EBV and HSV-1 shows more differences than similarities.

Although gp42 is critical for infection of B cells, it is dispensable for EBV entry into epithelial cells, which lack HLA. Indeed, several other herpesviruses do not require a specialized receptor-binding protein to trigger fusion (Heldwein and Krummenacher, 2008). So perhaps the real mystery is why in certain herpesviruses these specialized receptor-binding proteins are needed at all.

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For Structural Biology, Try Infrared Instead

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1D and 2D-IR spectroscopy was used to resolve the structural transition that is responsible for the gating mechanism of the influenza A M2 channel (Manor et al., 2009). This report constitutes a milestone in the development of both ATR-FTIR and 2D-IR spectroscopies as precise tools for structural biology.

Influenza A M2 proton channel is a homotetrameric integral membrane protein that plays an important role in the influenza A virus life cycle. Functionally, M2 protien is a pH-gated proton (H^+) channel, and details of the gating mechanism are not fully understood. In this issue, Manor et al. (2009) use a combination of sophisticated new vibrational spectroscopy methods, in combination with isotope labeling, to tackle and solve this long-standing problem of great biological relevance (i.e., the structural change that is responsible for the gating mechanism of the influenza A M2 channel [see Figure 4 of Manor et al. (2009)]). They show that the transmembrane helices turn by about 100° when switching from the closed to the